REMARKS

The Amendments

Claims 79-86 and 91 are amended to clarify the intended meaning. The amendments introduce no new matter and do not narrow the scope of the claims.

The Rejection of Claims 79-86, and 91 Under 35 U.S.C. § 112, second paragraph

Claims 79-86, and 91 are rejected as unclear because they are said to "read on luminescent proteins as cyan fluorescent protein." Similarly, claims 80, 82, 83, and 86 are said to erroneously imply that yellow fluorescent protein is a light emitting luciferase. While these claims appear to be definite and clear as originally presented, specifying a particular protein as being the "fluorescent or luminescent protein" recited in a previous claim, applicants have amended the claims to ensure clarity.

In the amended dependent claims, the element "fluorescent or luminescent protein" is first characterized as one of either a fluorescent or luminescent protein (*e.g.*, "first fluorescent or luminescent protein is a luminescent protein.") Second, the element is characterized as a particular type of such protein (*e.g.*, "the luminescent protein is a light-emitting luciferase protein.") It is respectfully submitted that the claim so amended is of identical scope as the originally presented version. Both versions are clear and definite.

Withdrawal of the rejection is respectfully requested.

The Rejection of Claims 11, 14-19, 21, 22, 25, and 56 Under 35 U.S.C. §103(a)

Claims 11, 14-19, 21, 22, 25, and 56 are rejected as unpatentable over Miyawaki[1] in view Jin[2]. This rejection is respectfully traversed.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. M.P.E.P. §2143. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck,* 947 F.2d 488, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991).

The subject matter of claims 11, 14-19, 21, 22, 25, and 56 is functional heterotrimeric G proteins which are capable of fluorescence energy resonance transfer (FRET).

Miyawaki is cited as teaching a system in which one fluorescent protein is attached to calmodulin and a second fluorescent protein is attached to M13, a calmodulin-binding polypeptide. The calmodulin and M13 are either fused together by a genetic fusion or they are two separate polypeptides. Upon binding of calcium to calmodulin, a change in FRET is observed, either due to a changed intramolecular conformation in the fusion protein or due to dimerization of the two separate polypeptides.

Jin is cited as teaching the interaction of G_α subunit with $G_{\beta\gamma}$ heterodimer to form a heterotrimer. The interaction is required for signal transduction in response to hormonal and chemical stimuli.

The Office Action alleges that it would have been obvious to the person of ordinary skill in the art to use the method of Miyawaki to attach a fluorescent donor and acceptor to the G_α and the G_β proteins as taught by Jin. The Office Action posits that one of ordinary skill in the art would have been motivated to combine the two teachings in order to "study the nature of the interactions between the G_α and the G_β proteins in response to various hormonal or sensory

1 *Nature, 388:* 882-887, 1997.
2 *Mol. Biol. Cell, 9:* 2949-2961, 1998.

signals ...." The alleged *prima facie* case of obviousness must fail, however, because it fails to fulfill the second prong of the obviousness test; there was no reasonable expectation of success.

The claims require that the heterotrimeric G protein be functional. The prior art did not provide any indication or suggestion that after modification of the alpha and beta subunits that the heterotrimer would remain functional.

Functional heterotrimeric G proteins are far more complex than the calmodulin system taught by Miyawaki. Miyawaki's system has three components: calmodulin, M13 polypeptide, and calcium. Calcium binds to the calmodulin and the fusion protein changes conformation[3], or the two separate polypeptides associate[4]. The heterotrimeric G proteins, in contrast, require far more interactions to be functional. First, after attachment of the FRET donor or receptor, the beta subunit must retain the ability to bind to the gamma subunit.[5] Second, after attachment of the FRET donor or receptor, the alpha subunit must retain the ability to bind to the beta-gamma heterodimer.[6] Third, after attachment of the FRET donor or receptor, the alpha subunit must retain GTPase activity.[7] Fourth, after attachment of the FRET donor and receptor, the heterotrimer must retain the ability to bind to the membrane bound G protein coupled receptor (GPCR) and to dissociate in response to the appropriate stimulus.[8]

Nothing in the teachings of Miyawaki or Jin provides any indication that all of these conditions could be met. Because of the complexity of the heterotrimeric G protein system, one of ordinary skill in the art would not have had a reasonable expectation of success that modifying the alpha and beta subunits of a heterotrimeric G protein with a FRET donor and receptor would yield a functional heterotrimeric G protein as claimed. Because of the inherent unpredictability of the system and because the prior art does not provide a reasonable expectation of success, the *prima facie* case must fail.

Withdrawal of the rejection is therefore requested.

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3 Miyawaki at Fig. 1A.
4 Miyawaki, page 886, col.. 2, first full paragraph
5 Specification at page 8, lines 20-21.
6 Specification at page 8, lines 20-21; Jin at page 2950, col. 1, lines 9-12.
7 Specification at page 1, lines 20-21; Jin at page 2950, col. 1, lines 12-15.
8 Jin at page 2949, col. 1, lines 1-5; page 2950, col. 1, lines 9-12.

The Rejection of Claims 13, 77-86, 89, 91-93 Under 35 U.S.C. §103(a)

Claims 13, 77-86, 89, 91-93 are rejected as unpatentable over Miyawaki in view of Jin and further in view of Xu. This rejection is respectfully traversed.

The rejected claims are directed to functional heterotrimeric G proteins that are (a) capable of FRET and have the fluorescent donor and receptor within 100 angstroms of each other (claim 13) or (b) are capable of BRET (claims 77-86, 89, 91-93).

The teachings of the primary (Miyawaki) and secondary (Jin) references are discussed above. Xu is cited as teaching that (a) FRET works well when the distance between the FRET donor and acceptor is between 10 and 100 angstroms, (b) that fluorescent and bioluminescent proteins may be used interchangeably in FRET and BRET, and (c) that BRET offers advantages over FRET for use in photo-responsive cells. Office Action at page 6.

For the same reasons discussed above with respect to claims 11, 14-19, 21, 22, 25, and 56, the *prima facie* case of obviousness of claims 13, 77-86, 89, 91-93 must fail. Briefly, the heterotrimeric G protein system requires retention of far more protein-protein interactions after modification than that required in the M13-calmodulin system taught by Miyawaki. In addition, the heterotrimeric G protein system requires retention of enzymatic activity. Retention of these many necessary functional properties was unpredictable. There was simply no reasonable expectation that one of skill in the art could successfully use the method of Miyawaki to modify the proteins taught by Jin (with or without the modifications of Xu) and retain the functionality of the heterotrimeric G protein as required by the claims. One of ordinary skill in the art simply could not have extrapolated from the teachings of Miyawaki, Jin, and Xu that the combination of teachings would yield a functional heterotrimeric G protein. Absent a reasonable expectation of success, the *prima facie* case must fail. *See* M.P.E.P. §2143.

Withdrawal of the rejection of claims 13, 77-86, 89, 91-93 is respectfully requested.

The Rejection of Claims 20, 23-24, 87-88, and 90 Under 35 U.S.C. §103(a)

Claims 20, 23-44, 87-88, and 90 are rejected as unpatentable over Miyawaki in view of Jin and further in view of Medina and Wall. This rejection is respectfully traversed.

Claims 20, 23-24, 87-88, and 90 are directed to functional heterotrimeric G proteins that are capable of FRET or BRET in which an amino acid sequence for a FRET or BRET donor or acceptor is inserted in a helical domain of an alpha subunit or at the N-terminus of the beta subunit.

The teachings of the primary (Miyawaki) and secondary (Jin) references are discussed above. Medina is cited as teaching that the two termini of the alpha subunit are important for function and that internal residues 57-181 could be replaced with homologous residues of alpha i2 subunit and retain function. Wall is also cited as teaching (a) that the two termini of the alpha subunit are important for function, (b) that the beta and gamma subunits are tightly bound, and (c) that the N-terminus of the beta subunit is open and forms an extended helical polypeptide chain pointing toward the N-terminus of the alpha subunit. The teachings of Medina and Wall are said to render it obvious that the fluorescent donor should be inserted in the helical loop of the alpha protein and the fluorescent acceptor protein should be inserted at the N terminus of the beta protein.

Medina and Wall do not remedy the deficiencies in the primary and secondary references in rendering the invention obvious.[9] None of Miyawaki, Jin, Medina, or Wall provided the art with a reasonable expectation of success that the alpha and beta subunits of heterotrimeric G

9 Applicants do not concede the alleged implications of the teachings of Medina and Wall posited in the rejection.

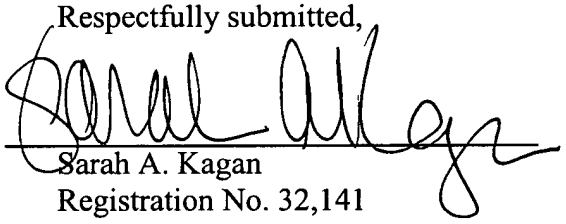
proteins could be modified with FRET and/or BRET donor and acceptor sequences and retain functionality. As discussed above, the heterotrimeric G protein system involves many necessary protein-protein interactions and enzymatic activity. Such a highly constrained system could not have been predicted to retain functionality simply on the basis of extrapolation from the simple system of calmodulin and M13 polypeptide. Because one of skill in the art would not have had a reasonable expectation of success, the *prima facie* case of obviousness fails. *See* M.P.E.P. §2143.

Withdrawal of this rejection is respectfully requested.

A speedy allowance of all pending claims is respectfully requested.

Respectfully submitted,

Date: February 24, 2005

By: 

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